Prdx6 Deficiency Ameliorates DSS Colitis: Relevance of Compensatory Antioxidant Mechanisms

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Abstract: Background and Aims An imbalance between cellular antioxidant defence system[s] and reactive oxygen species [ROS]-driven oxidative stress has been implicated in the pathogenesis of inflammatory bowel disease. Peroxiredoxin [PRDX] 6 contributes to an appropriate redox balance by clearing ROS and reducing peroxidized membrane phospholipids. We here studied the role of PRDX6 in acute and chronic dextran sodium sulphate [DSS]-induced colitis. Methods To investigate the impact of PRDX6 on intestinal inflammation, we used wild type [WT], Prdx6 knock-out mice [Prdx6-/-] and transgenic mice [Prdx6tg/tg], overexpressing Prdx6. Acute and chronic colitis was induced by DSS in WT, Prdx6-/- and Prdx6tg/tg mice. Colitis was evaluated by endoscopy, colon length, histopathological assessment and myeloperoxidase [MPO] activity. Changes in mRNA and protein expression of pro-inflammatory cytokines and antioxidant enzymes were evaluated by real-time quantitative polymerase chain reaction [RT-qPCR] and western blot. Total glutathione [GSH] levels in colon samples were determined. Results Prdx6-/- mice exposed to acute and chronic DSS showed a significant decrease in the clinical parameters and in colonic expression of pro-inflammatory cytokines compared with WT mice. mRNA expression of antioxidant enzymes in colon samples was significantly increased in Prdx6-/- compared with WT mice exposed to acute and chronic DSS. In addition, total GSH levels were increased in Prdx6-/- mice treated with DSS in comparison with WT. Overexpression of Prdx6 did not significantly influence acute and chronic colitis. Conclusions Our data indicate that a lack of the antioxidant enzyme PRDX6 protects against the development of acute and chronic experimental colitis and is associated with increased expression and function of other antioxidant enzymes, suggesting effective compensatory mechanisms.

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Prdx6 deficiency ameliorates DSS colitis: relevance of compensatory antioxidant mechanisms

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Short title: Lack of Prdx6 ameliorates DSS colitis

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Abbreviations

BCA, bicinchoninic acid assay; colonic lamina propria mononuclear cells (LPMC); CD, Crohns's disease; DSS, dextran sodium sulphate; GSH, reduced glutathione; IBD, inflammatory bowel disease; intestinal epithelial cells, (IEC); MEICS, murine endoscopic index of colitis severity; MPO, Myeloperoxidase; NO, Nitric oxide; ROS, reactive oxygen species; Tg, transgenic; UC, ulcerative colitis; UV, ultraviolet

Abstract

Background

An imbalance between cellular antioxidant defense system(s) and reactive oxygen species (ROS)-driven oxidative stress has been implicated in the pathogenesis of inflammatory bowel disease. Peroxiredoxin (PRDX) 6, contributes to an appropriate redox balance by clearing ROS and reducing peroxidized membrane phospholipids.

Aims

We here studied the role of Prdx6 in acute and chronic DSS-induced colitis.

Design

To investigate the impact of PRDX6 on intestinal inflammation, we used wild type (WT), *Prdx6* knock-out mice (*Prdx6*$^{-/-}$) and transgenic mice (*Prdx6*$^{tg/tg}$), overexpressing *Prdx6*. Acute and chronic colitis was induced by dextran sulfate sodium (DSS) in WT, *Prdx6*$^{-/-}$, and *Prdx6*$^{tg/tg}$ mice. Colitis was evaluated by endoscopy, colon length, histopathological assessment, and myeloperoxidase (MPO) activity. Changes in mRNA and protein expression of pro-inflammatory cytokines and antioxidant enzymes were evaluated by RT-qPCR and western blot. Total GSH levels in colon samples were determined.

Results

Prdx6$^{-/-}$ mice exposed to acute and chronic DSS showed a significant decrease in the clinical parameters and in colonic expression of pro-inflammatory cytokines compared to WT mice. mRNA expression of antioxidant enzymes in colon samples was significantly increased in *Prdx6*$^{-/-}$ compared to WT mice exposed to acute and chronic DSS. In addition, total GSH

levels were increased in *Prdx6*$^{-/-}$ mice treated with DSS in comparison to WT. Overexpression of *Prdx6* did not significantly influence acute and chronic colitis.

Conclusion

Our data indicate that a lack of the antioxidant enzyme PRDX6 protects against the development of acute and chronic experimental colitis and is associated with increased expression and function of other antioxidant enzymes suggesting effective compensatory mechanisms.

Keywords

IBD, colitis, ROS, Prdx6, antioxidant enzymes

Introduction

The major forms of inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing inflammatory disorders of the gastrointestinal (GI) tract causing a life-long burden on affected patients (1). Both disorders have a multifactorial pathogenesis with three main factors involved in their pathogenesis: environmental exposure (e.g. to gut microbiota and microbial pathogens), genetic susceptibility (e.g. predisposing factors affecting the gut barrier function) and consecutive dysregulation of the immune system (on the innate and adaptive level) (2-3).

The mechanisms by which imbalances of the immune system initiate and perpetuate intestinal damage and inflammation are not entirely clear. However, inflammatory conditions lead to an increased oxidative burden due to the release of reactive oxygen species (ROS) by activated macrophages and neutrophils as a part of the body's defense mechanisms (4). Low levels of ROS have a protective effect by activating protective signaling pathways against inflammation and by modulating the immune-mediated attack against extrinsic pathogens. Conversely, high levels of ROS create oxidative stress within the cells and, in case of chronic exposure, changes in the genome, such as single strand DNA breaks, sugar moiety modifications, and deamination and adduction of bases (5), leading to a loss of homeostasis (6).

Several studies have established a direct relation between oxidative stress and IBD pathogenesis in both, human subjects and animals subjected to experimental colitis. Clinical studies showed an increase of ROS as well as biomarkers of oxidative injury, including lipid peroxidation products and protein modifications, in the intestinal mucosa of patients with either UC or CD (7-8). These highly cytotoxic molecules contribute to tissue damage in IBD (9). In line with this, Barbosa *et al.,* (10) showed a correlation between the activity of free radicals in the intestinal mucosa of IBD patients, and the clinical disease activity. To maintain adequate ROS levels, a balance between oxidant and antioxidant mechanisms is necessary. A

depletion of antioxidant levels in the intestinal mucosa and in peripheral red blood cells has been observed in IBD patients (11-13). Genetic polymorphisms in antioxidant enzymes are associated with altered enzyme activity and a higher risk of developing IBD (14). In animal models of IBD, multiple studies demonstrated an increased formation of ROS in the colonic mucosa (15-16). In line with this, experimental colitis is associated with a decrease in the levels of endogenous antioxidants in colonic tissue (17). Genetic deletion of ROS-detoxifying enzymes increases the susceptibility towards tissue destruction (18), whereas an overexpression of antioxidant enzymes resulted in attenuation of colitis in mice (19-20).

Among the most important antioxidant proteins is the peroxiredoxin (PRDX) family. PRDX6, a bifunctional 25-kDa protein, is the only member of PRDXs that confers both GSH peroxidase and phospholipase A_2 activities (21). Despite intensive investigations, the role of PRDX6 in inflammation is still controversial and both protective (experimental autoimmune encephalomyelitis (22), UV light exposure (23) wound healing in the skin (24) and hepatocellular injury in an ischemia-reperfusion model (25)). as well as deleterious effects (e.g. higher loss of dopaminergic neurons in a model of Parkinsons disease(26)). In order to elucidate the impact of PRDX6 in intestinal inflammation and, for the first time address its role in IBD, we induced acute and chronic colitis in *Prdx6* deficient or overexpressing mice.

Materials and Methods

Patients

Colon and Ileum samples of IBD patients and controls were obtained by colonoscopy as described previously (27)

Animals

Prdx6 knockout mice (B6.129-Prdx6tm1Pgn/Pgn, here termed *Prdx6*$^{-/-}$), with targeted disruption of the *Prdx6* gene (28-29), *Prdx6* transgenic (C57BL/6J-Tg(Prdx6)153Pgn/Pgn, here termed *Prdx6*$^{tg/tg}$) mice expressing the *Prdx6* sequence of the 129X1/SvJ mouse strain (30-31), and wild-type (WT) mice in the C57BL/6J genetic background were purchased from Jackson Laboratory (Bar Harbor, ME). The 129X1/SvJ sequence for *Prdx6* contains an amino acid variant at position 124 encoding aspartic acid instead of alanine, leading to moderate overexpression of Prdx6 (30). *Prdx6*$^{-/-}$ and *Prdx6*$^{tg/tg}$ animals, developped normally, with no differences in survival rate and breeding performance in comparison to wildtype controls. Mice were kept under specific pathogen-free (SPF) conditions in the animal facility of the University Hospital Zurich with access to food and water *ad libitum*. All animal experiments were performed in accordance with institutional and state guidelines under animal experiment license No. 206/2007.54/2011.

Induction of acute and chronic DSS-induced colitis

Female mice received 2% DSS (MW 36 000–50 000, MP Biomedicals, Santa Ana, USA) in drinking water for 7 days for induction of acute DSS colitis and five 7-day cycles of 2% DSS interspaced with 10-day recovery phases with normal drinking water for induction of chronic DSS colitis. After the last DSS cycles animals were kept on normal drinking water for another 4 weeks to investigate the then established chronic intestinal inflammation. During induction of colitis, clinical symptoms were recorded daily (weight change, animal appearance, stool appearance). At the end of the experiment (day 7 or 105), colitis severity was assessed by

endoscopy and tissues sample were collected. Results represent two independent experiments with a total of n = 12 per group.

Endoscopic assessment and scoring

Prior to endoscopy, animals were anaesthetized i.p. with 90–120 mg ketamine (Vetoquinol AG, Bern 169 Switzerland) and 8 mg xylazine (Bayer, Lyssach, Switzerland) per kg body weight. The distal 3 cm of the colon and the rectum were examined with a Karl Storz Tele Pack Pal 20043020 (Karl Storz Endoskope, Tuttlingen, Germany). Endoscopic score was evaluated according to a scoring system (murine endoscopic index of colitis severity (MEICS)) as described previously (32).

Histological assessment and scoring

Histological assessment was performed on 5-µM-thick sections of the distal colon, stained with hematoxylin and eosin (H&E). Histological score was assessed by two independent blinded investigators as described previously (33):Epithelium (E) 0: normal morphology; 1: loss of goblet cells; 2: loss of goblet cells in large areas; 3: loss of crypts; 4: loss of crypts in large areas. Infiltration (I) 0: no infiltrate; 1: infiltrate around crypt basis; 2: infiltrate reaching to L. muscularis mucosae; 3: extensive infiltration reaching the L. muscularis mucosae and thickening of the mucosa with abundant oedema; 4: infiltration of the L. submucosa. The total histological score represents the sum of the epithelium and infiltration score in the distal and in the proximal colon, and thus ranges from 0 to 16 (total score = $(E + I)_{distal} + (E + I)_{proximal}$).

Formatiert: Italienisch (Italien)

Myeloperoxidase (MPO) activity

Colon tissue samples were mechanically homogenized in 50 mM phosphate buffer containing 0.5 % hexadecyltrimethylammonium bromide (Sigma-Aldrich, St. Louis, MO, USA) the and

then subjected to three freeze-thaw cycles. Samples were centrifuged at 17,000 g for 2 min; and 20 µl of each supernatant was transferred in duplicate to a 96-well plate and 280 µl of 0.02 % dianisidine (in 50 mM phosphate buffer and 0.0005 % H2O2; Sigma-Aldrich) were added. The absorbance was measured at 460 nm after 20 min incubation at room temperature, Protein was quantified by bicinchoninic acid assay (BCA; Pierce Biotechnology, Rockford, IL, USA) and the MPO activity (arbitrary units, U) was calculated as mean absorbance at 460 nm/incubation time/protein concentration (µg/20 µl).

Intestinal epithelial cells (IEC) and colonic lamina propria mononuclear cells (LPMC) isolation

2/3 of the Colon were cut lengthwise, freed from residual faeces and cut into small pieces and transferred to Hank's balanced salt solution (HBSS; PAA, Linz, Austria) supplemented with 2 mM of EDTA and stirred for 30 min at 37°C. Mucosal pieces were removed by passing the slurry over a coarse mesh (400 µm; Carl Roth GmbH, Karlsruhe, Germany). The sieve residue was flushed back to the tube with fresh HBSS, vigorously shaken 10 times and vortexed for 1 min for 2 times. Mucosal pieces were partitioned again from detached cells by filtration through mesh (70 µm; Falcon, Corning, MA, USA). The shake and vortex step was repeated 2 times The combined filtered fractions were collected and centrifuged at 400 g for 7 min. The cell pellet – IECs -was re-suspended in RNA isolation buffer and stored at -80°C until RNA isolation. For the LPMC isolation an enzyme cocktail (Collagenase V, 0.425mgl/ml, Sigma; Collagenease D,0.75 mg/ml, Roche; Dispase, 1mg/ml Gibco; DNase 20 µg/ml, Roche) was added to the supernatant followed by digestion for 15-25 min at 37°C with shaking. The cell suspension was filtered through a cell strainer (70 µm; Falcon, Corning, MA, USA). The filtered fraction was centrifuged at 400 g for 7 min, the cell pellet –LMPC – resuspended in RNA isolation buffer and stored at -80°C until RNA isolation.

RNA isolation and Real Time-qPCR

RNA isolation and cDNA synthesis from human biopsies was performed as described previously (27). For RNA isolation from mouse samples colon samples of WT (n=12), *Prdx6*$^{-/-}$ (n=12) and *Prdx6*$^{tg/tg}$ (n=12) mice in RLT buffer, were homogenized mechanically and total RNA was purified with the RNeasy Mini Kit in a QIACUBE operating facility (Qiagen, Venlo, Netherlands) following the manufacturer`s recommendations. 1µg total RNA was reverse-transcribed and amplified using the transcription kit from Life Technology (Carlsbad, CA, USA). The following Taqman® assays (Life Technology, Carlsbad, CA, USA) were used in combination with TaqMan® Fast Universal PCR Master Mix: *PRDX6* (Hs00705355_s1), *VIL1* (Hs00200229_m1), *Tnfa* (Mm99999068_m1), *Nos2* (Mm01309893_m1), *Ifng* (Mm00801778_m1), *Il6* (Mm00446190_m1), *Il1b (Mm01336189_m1)*, *Prdx3* (Mm00545844_m1), *Prdx4* (Mm00450261_m1), *Nrf2* (Mm00477786_m1), *Gss* (Mm00515065_m1), *Gclm* (Mm00514996_m1), and *Actb1* (4352341E). Amplifications were performed in triplicates, and data were normalized to VIL1 levels in human biopsies and to *Actb1* levels in murine samples.

Immunoblotting

Total protein was extracted from the colon by lysing homogenized tissue in RIPA buffer (0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40) supplemented with protease inhibitors (Roche Diagnostics, Mannheim, Germany) and quantified using the BCA method. For each group, a total of 30 µg of protein was transferred to a nitrocellulose membrane after electrophoretic separation. The membrane was pre-hybridized in 5 g/L skim milk or BSA for 1 h. The nitrocellulose membrane was then incubated overnight with Prdx3, Prdx4, Prdx6, Nrf2, Gss, Gclm and Gclc primary antibody. After washing in TBS, the appropriate secondary antibody conjugated to HRP was added, and the membrane was incubated at ambient temperature for 1 h. After washing in TBST again, the proteins were visualized using an ECL

or ECL PLUS detection kit (Amersham, Velizy-Villacoublay, France). β-Actin was used as an internal reference control.

Determination of glutathione content

Total glutathione (GSH and reduced GSSG) levels were determined according to a modification of the Tietze recycling assay as described by Rahman et al.(34). In brief, deproteinized cytosolic extracts of tissue samples were obtained by mechanical homogenization in extraction buffer (0.1% Triton X-100 and 0.6% sulfosalicylic acid in 0.1M potassium phosphate buffer with 5 mM EDTA disodium salt, pH 7.5). After centrifugation at 2000 rpm for 5 minutes at 4°C, the resultant supernatant was added to 120 µl of DTNB and glutathione reductase solution. 30 seconds after, 30 µl of β-NADPH was added. The absorbance of supernatant was measured at 412 nm against a reagent blank. The amount of GSH was expressed as µM/mg protein.

Statistical analysis

Statistical analysis was performed with the statistical software package SPSS Version 22 using the Mann-Whitney rank sum test for non-parametrically distributed samples. Differences were considered significant at $p < 0.05$ (*), highly significant at $p < 0.01$ (**), and very highly significant at $p < 0.001$ (***).

Results

Prdx6$^{-/-}$ mice are more resistant to DSS-induced acute and chronic colitis

To characterize the function of PRDX6 during intestinal inflammation, WT and Prdx6$^{-/-}$ mice at 8 weeks of age were administered 2% DSS in their drinking water for 7 days to induce inflammation in the large intestine. Colitis was characterized by weight loss, bloody diarrhea and inflammatory cell infiltration as previously described (35). On challenge with DSS, the onset of weight loss occurred earlier in WT mice, with 10.3 +/- 1.4 % weight loss in WT mice on day 6 in contrast to 5.4 +/- 1.5 % weight loss in *Prdx6*$^{-/-}$ mice ($p = 0.0336$; Figure 1A). Further, on day 7 WT mice displayed significantly more severe body weight loss in comparison to *Prdx6*$^{-/-}$ mice (WT 11.7 +/- 1.3% vs 6.3 +/- 1.7% in Prdx6$^{-/-}$; $p = 0.0315$; Figure 1A). Consistently, at autopsy, WT DSS mice showed more pronounced shortening of the large intestine in comparison to *Prdx6*$^{-/-}$ DSS mice (6.3 cm vs 7.9 cm $p < 0.0001$; Figure 1B). The reduced susceptibility of *Prdx6*$^{-/-}$ mice to acute DSS-induced colitis was confirmed when analyzing further parameters indicative of intestinal inflammation: MPO activity was lower in colon samples from the *Prdx6*$^{-/-}$ DSS group in comparison to the WT DSS group (145.5 U vs 742.5 U $p < 0.0001$; Figure 1C), and the colonoscopy score was lower in *Prdx6*$^{-/-}$ DSS mice in comparison to the WT DSS group (5.1 vs 2 $p = 0.0049$) (Figure 1D). Histological analysis of H&E stained colon tissue sections from WT DSS mice showed significantly more mucosal damage, loss of goblet cells and inflammatory cell infiltrate than *Prdx6*$^{-/-}$ DSS mice (12.1 vs 5.9 $p < 0.0001$) (Figure 1E).

In contrast to the marked protection from body weight loss in *Prdx6*$^{-/-}$ mice during acute colitis, no difference was found for body weight development between *Prdx6*$^{-/-}$ and WT mice after five cycles of DSS treatment (Figure 2A). Nevertheless, colon length of WT mice was significantly stronger reduced by inflammation than in *Prdx6*$^{-/-}$ mice (7.1 cm vs 8.4 cm $p = 0.0025$; Figure 2B), and MPO activity was lower in *Prdx6*$^{-/-}$ mice when in comparison to the control group (96.25 U vs 153.2 U; $p = 0.0486$; Figure 2C). Endoscopic analysis of the colon

as evaluated by murine endoscopic index of colitis severity (MEICS) revealed more severe inflammation in DSS treated WT mice in comparison to the *Prdx6*$^{-/-}$ DSS group (6.5 vs 3.6 p = 0.0230; Figure 2D). Consistently, histological analysis demonstrated more severe inflammation in WT than in *Prdx6*$^{-/-}$ mice: more mucosal damage, loss of goblet cells and inflammatory cell infiltration was observed in WT mice with an average score of 12.8 in WT vs 8.7 in *Prdx6*$^{-/-}$ mice (p = 0.0236; Figure 2E). Taken together, our results demonstrate that genetic ablation of *Prdx6* renders mice less susceptible to DSS-induced acute and chronic colitis.

Loss of *Prdx6* decreases the expression of pro-inflammatory cytokines in DSS-induced acute and chronic colitis

To further study the impact of loss of *Prdx6* on intestinal inflammation, the expression of mRNAs coding for TNF-α, iNOS,IFN-γ IL-6 and IL-1β was analyzed. In acute colitis, *Prdx6*$^{-/-}$ DSS mice showed significantly reduced expression of *Tnfa* (2.3 fold, p = 0.0135), *Nos2* (4.6 fold, p = 0.0185), *Ifng* (26.2 fold, p = 0.0477), *IL6*(3.9 fold, p = 0.0184) and *IL1b* (6.6 fold, p = 0.0109) in comparison to the WT DSS animals (Figure 1F). Consistently, in DSS-induced chronic colitis, there was a significant decrease in the expression level of *Tnfa (7* fold, p = 0.0450), *Nos2* (4.2 fold, p = 0.0156), *Ifng* (5 fold, p = 0.0012) *IL6* (7.6 fold, p = 0.0228) in *Prdx6*$^{-/-}$ DSS mice in comparison to WT DSS animals (Figure 2F).

Prdx6 is downregulated in murine experimental colitis but overexpressed in human colonic biopsies of IBD patients

To study how endogenous Prdx6 expression is regulated during colitis, we determined PRDX6 protein expression by Western blotting. Both wildtype as well as *Prdx6*$^{tg/tg}$ animals show significant downregulation of PRDX6 in the acute DSS colitis model (p = 0.0001 and p

307 = 0.0136, Figure 3A, B). A similar downregulation was observed in the chronic model (Data
308 not shown). We further analysed the mRNA expression of Prdx6 in colonic and ileal biopsies
309 of IBD patients and controls. Interestingly, we observed a significant increase in Prdx6
310 mRNA levels in colonic biopsies of IBD patients taken from affected tissue (Figure 3C) while
311 ileal biopsies showed a tendency towards downregulation of Prdx6 (Figure 3D).
312
313 **Loss of *Prdx6* induces the expression of other peroxiredoxins and antioxidant enzymes in**
314 **mice exposed to acute and chronic DSS**
315 In order to understand how loss of *Prdx6* exerts a protective effect during intestinal
316 inflammation, we next analyzed the mRNA expression of other Prdx-family members such as
317 PRDX3 and PRDX4 and antioxidant enzymes such as NRF2, GSS and GCLM in WT and
318 *Prdx6*$^{-/-}$ mice exposed to acute or chronic DSS treatment. On challenge with acute DSS
319 adminitstration, *Prdx6*$^{-/-}$ mice showed a significant increase in mRNA expression of *Prdx3*
320 (2.31 fold, p = 0.0306), *Prdx4* (1.68 fold, p = 0.0110), *Nrf2* (1.92 fold, p = 0.0296) and *Gss*
321 (1.69 fold, p = 0.041) in comparison to the WT DSS group (Figure 4A). The same effects
322 were also observed upon chronic DSS-treatment: *Prdx6*$^{-/-}$ mice treated with DSS showed an
323 increase in mRNA expression of *Prdx3* (1.5 fold, p = 0.0299), *Prdx4* (1.74 fold, p = 0.0219),
324 *Nrf2* (1.92 fold, p = 0.0060), *Gss* (2.62 fold, p < 0.0001), and *Gclm* (1.76 fold, p = 0.0191)
325 (Figure 4B). However, the expression level of *Prdx5* did not differ between *Prdx6*$^{-/-}$ and WT
326 mice (data not shown).
327 Results at mRNA level were confirmed by immunoblotting. In the acute DSS model, there
328 was a significant increase in the expression level of *Prdx3* (2.4 fold, p < 0.0001), *Prdx4* (1.8
329 fold, p = 0.0005), *Gss* (1.5 fold, p = 0.099), *Gclm* (1.5 fold, p < 0.0001) and *Gclc* (2.1 fold, p
330 = 0.0057) (Figure 4C). In colonic mucosa of mice exposed to chronic DSS, the expression
331 level of *Prdx3* (2.3 fold, p < 0.0001), *Prdx4* (1.5 fold, p < 0.0001) and *Gclm* (2.3 fold, p <
332 0.0001) was significantly increased (Figure 4D).

To study functional consequences of altered *Gss* and *Gclm* mRNA expression, we determined total GSH levels in colon homogenates of WT and Prdx6$^{-/-}$ mice exposed to DSS. In both, the acute and the chronic DSS model, total GSH levels were increased in *Prdx6*$^{-/-}$ mice in comparison to the WT DSS group (2.47 fold, p = 0.0246) and (1.87 fold, p = 0.0001; Figure 4E and 4F).

Increased expression of Peroxiredoxins and Nrf2 in LPMC but not in IEC

To determine which cellular compartment was responsible for protection in Prdx6 knockout mice, we evaluated the mRNA expression level of antioxidant enzymes (Prdx3, Prdx4, Nrf2, Gss and Gclm) in epithelial cells and in lamina propria mononuclear cells isolated form *Prdx6*$^{-/-}$ and WT mice exposed to acute DSS colitis. No differences were observed in the expression levels of these antioxidant enzymes in epithelial cells (Figure 5A). However, we saw significant increases in the mRNA expression levels of Prdx3 (fold increase 4.3, p = 0.026), Prdx4 (fold increase 1.7, p = 0.016) and Nrf2 (fold increase 2.8, p = 0.0217) in lamina propria mononuclear cells (Figure 5B).

Overexpression of *Prdx6* does not influence the intestinal inflammation in the acute and chronic DSS model

Given the positive effect of the genetic ablation of *Prdx6* in DSS induced colitis, we sought to investigate whether the overexpression of *Prdx6* aggravates the outcome of colitis models. For this purpose, we used transgenic mice (*Prdxg*$^{tg/tg}$) expressing the *Prdx6* sequence of the 129X1/SvJ mouse strain, and subjected them to acute and chronic DSS colitis. The 129X1/SvJ sequence contains an amino acid variant at position 124 encoding aspartic acid instead of alanine, leading to moderate overexpression of Prdx6 (30). At day 7 of acute DSS treatment, animals were euthanized and body weight and colon length were measured. No differences in these parameters were found between *Prdx6*$^{tg/tg}$ and WT mice treated with DSS

(Figure 6A, and B). Consistently, MPO activity, endoscopic score and histological score did not differ between *Prdx6*$^{tg/tg}$ DSS and WT DSS animals (Figure 6C-E). Likewise, we found no differences between *Prdx6*$^{tg/tg}$ and WT mic in the chronic DSS colitis model: body weight loss (Figure 7A), colon length (Figure 7B), and MPO activity (Figure 7C) did not show any significant difference between *Prdx6*$^{tg/tg}$ DSS and WT DSS animals. Consistently, endoscopic (Figure 7D) and histologic (Figure 7E) evaluation did not show any difference between *Prdx6*$^{tg/tg}$ DSS and WT DSS mice.

Cytokine expression in the colon differs in acute and chronic colitis

To study the effect of *Prdx6* overexpression on intestinal inflammation, mRNA expression of pro-inflammatory mediators (TNF-α, iNOS and IFN-γ) was assessed by RT-qPCR in colon tissue samples. In acute colitis, mRNAexpression of *Tnfa* and *Ifng* in *Prdx6*$^{tg/tg}$ DSS mice were not significantly different from WT DSS mice (Figure 6F). In chronic colitis, however, *Prdx6*$^{tg/tg}$ DSS mice showed significantly lower expression of *Tnfa (*1.8 fold, p = 0.0190) and *Ifng* (10 fold, p = 0.0386) in comparison to WT DSS mice (Figure 7F).

Overall, our findings show that although loss of *Prdx6* protected from DSS-induced colitis, Prdx6 overexpression does not aggravate intestinal inflammation. In the chronic colitis model even lower expression of pro-inflammatory cytokines can be observed.

Levels of peroxiredoxins and antioxidant enzymes in colons of WT and *Prdxg*$^{tg/tg}$ mice exposed to chronic DSS

Our results suggested that *Prdx6* overexpressiion did not influence the severity of DSS-induced acute and chronic acoltis. However, in the chronic model, we saw a significant decrease in the expression level of TNFα and IFNγ. We therefore sought to investigate whether the overexpression of *Prdx6* in the chronic model is associated with increased expression of other peroxiredoxins and antioxidant enzymes. For this purpose, we analyzed

385 the mRNA expression of Prdx3, Prdx4, Nrf2, Gss and Gclm in WT and *Prdx6*$^{tg/tg}$ mice
386 exposed to chronic DSS treatment. The results showed a significant increase in mRNA
387 expression of Prdx3 (2.9 fold, p = 0.032), Nrf2 (1.52 fold, p = 0.005) and Gss (2 fold, p =
388 0.0034) in comparison to the WT DSS group (Figure 8).
389

Discussion

As an antioxidant enzyme, PRDX6 controls the reactive oxygen species (ROS) level within cells (36). Intestinal inflammation is accompanied by massive ROS production by activated macrophages and neutrophils, which contributes to inflammation associated tissue destruction (4), hence antioxidant enzymes might play an important role during intestinal inflammation. We therefore hypothesized that knockout of *Prdx6* would lead to enhanced susceptibility to DSS-induced acute and chronic intestinal inflammation, whereas overexpression of *Prdx6* would protect against DSS-induced colitis. Surprisingly, our results showed that genetic ablation of *Prdx6* did not aggravate inflammation, but in contrast, protected animals from DSS-induced acute and chronic colitis. On challenge with DSS, *Prdx6*$^{-/-}$ mice, in comparison to WT, showed significantly less inflammation as indicated by clinical parameters and mRNA levels of pro-inflammatory cytokines. Lower inflammation was accompanied by an increase in mRNA expression of eantioxidant enzymes such as *Prdx3*, *Prdx4*, *Nrf2*, *Gss* and *Gclm*. Consistently, total GSH levels were increased in *Prdx6* deficient mice in comparison to WT mice. Taken together, this study shows that targeted disruption of *Prdx6* ameliorates colitis, associated with an increase in expression of other antioxidant enzymes and a rise in GSH levels suggesting effective compensatory mechanisms.

Increased neutrophil infiltration in the colon is one of the hallmarks of pathophysiology in IBD and in the model of DSS induced experimental colitis. MPO is predominantly expressed by neutrophils thus assessment of MPO activity serves as a useful biomarker for neutrophil infiltration. Our results showed that DSS administration, as expected, increased colonic MPO activity, indicating a significant rise in neutrophil infiltration in the DSS colitis models in WT animals. Surprisingly, our data showed a significantly lower increase in MPO activity, in *Prdx6*$^{-/-}$ mice exposed to both acute and chronic DSS administration in comparison to WT animals. This lower neutrophil infiltration in *Prdx6*$^{-/-}$ animals might even be accompanied by lower ROS production, as loss of *Prdx6* has been show to impair NADPH oxidase activity

(37). In line with the observed differences in neutrophil infiltration, higher MEICS and histological scores indicated severe tissue damage in WT DSS animals while *Prdx6*$^{-/-}$ DSS animals showed significantly lower scores.

In IBD, pro-inflammatory cytokines play an important role in the development and progression of the intestinal immune response. Accumulating data on animal models and human studies, show an increase in inflammatory cytokines, in particular TNF-α and a direct relationship between cytokine expression and extent of inflammation. TNF-α activates neutrophils and macrophages, in addition to its induction of IFN-γ production. In this study, on challenge with DSS, the mRNA expression of TNF-α, IFN-γ; Il-6 and IL1β was decreased in *Prdx6*$^{-/-}$ mice. Nitric oxide (NO), a key biomarker of oxidative stress, is synthesized by inducible nitric oxide synthase (iNOS) which is thought to be the main producer of NO in IBD (38). During inflammation, iNOS is activated and it is induced by cytokines including TNF-α, and IFN-γ. Our results showed a significant decrease in the mRNA expression level of iNOS in *Prdx6*$^{-/-}$ mice exposed to acute and chronic DSS. Taken together, our results showed that genetic ablation of *Prdx6* conferred protection against DSS-induced acute and chronic colitis.

As lack of Prdx6 had a beneficial effect in the investigated DSS colitis models we next studied regulation of endogenous Prdx6 protein expression upon induction of colitis. In both the acute as well as the chronic DSS model wildtype and *Prdx6*$^{tg/tg}$ animals showed a significant downregulation of PRDX6 in line with the downregulation of PRDX6 in the acute DSS colitis model reported by Naito *et al.* (39). These findings suggest that no PRDX6 expression is beneficial during intestinal inflammation as downregulation of Prdx6 seems to be a physiological response during in murine colitis models.

In human biopsies the results are more complex. In contrast to the murine data, colonic samples showed significant upregulation of Prdx6 mRNA levels in areas affected by IBD.

Ileal samples on the other hand, showed a non-significant downregulation of Prdx6 mRNA expression in IBD affected tissue similar to the regulation observed in murine samples. Previously, increased expression of PRDX6 particularly in epithelial cells of ileal samples of CD patients has been observed (40). These findings suggest that more detailed studies investigating the regulation of Prdx6 at different stages of inflammation and in different areas of the intestine are necessary to clarify the role of Prdx6 in human IBD.

To address whether downregulation or lack of Prdx6 during colitis acts beneficial by allowing for compensatory upregulation of other Peroxiredoxins we determined the mRNA levels of three other 2-Cys peroxiredoxins (*Prdx3*, *Prdx4* and *Prdx5*) in colon samples of *Prdx6*$^{-/-}$ and WT mice exposed to acute and chronic DSS. To date there is no report describing the role of *Prdx3* and *Prdx4* in IBD, however accumulating data indicate a protective role of these antioxidant enzymes in inflammation. Li *et al.,* (41) showed that intratracheal instillation of LPS triggered higher ROS levels in macrophages and more oxidative damage to DNA and proteins in *Prdx3* KO mice compared to control animals. *Prdx4* was shown to protect against nonalcoholic steatohepatitis, type 2 diabetes mellitus and the metabolic syndrome by reducing oxidative stress-induced injuries (42). In this study, the mRNA expression levels of *Prdx3* and *Prdx4* were significantly increased in *Prdx6*$^{-/-}$ mice in comparison to WT mice. It is therefore very likely that the increased mRNA levels of *Prdx3* and *Prdx4* in the colon might compensate for the lack of *Prdx6* and lead to enhanced antioxidant defense against DSS-induced oxidative stress in the absence of *Prdx6*. In line with our results, two previous reports found an increase of *Prdx3* and *Prdx4* in *Prdx6*$^{-/-}$ mice in a hepatic ischemia/re-perfusion injury model and in cigarette smoke-mediated lung inflammatory response (25, 43). Taken together, the increase of *Prdx3* and *Prdx4* in the colon of *Prdx6*$^{-/-}$ mice suggests a compensatory response by other Prdx enzymes in response to acute and chronic DSS exposure.

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a redox-sensitive transcription factor orchestrating key cellular antioxidant response mechanisms. It activates a wide variety of genes encoding cytoprotective phase II detoxification and antioxidant enzymes such as glutathione synthetase (GSS), and glutamate-cysteine ligase (GCL) (44-47) via the antioxidant-responsive element promoter (44, 48). It has been documented that the lack of Nrf2 leads to a higher susceptibility to DSS-induced colitis (49) and azoxymethane/DSS colitis associated colorectal carcinoma (50). Additionally, several studies showed that the application of Nrf2 activating substances ameliorated DSS-induced acute colitis (51-52). In this study, the mRNA expression of *Nrf2*, *Gclm* and *Gss* was increased in *Prdx6*$^{-/-}$ animals exposed to DSS. Nrf2 can affect glutathione (GSH) synthesis, one of the most critical cellular antioxidants, by increasing the expression of *Gclc* and *Gclm* subunits and *Gss* (53-54). GSH is associated with reduced inflammation (55), inhibition of tumor cell growth (56), and prevention of apoptosis (57). As shown in our study, total GSH levels were increased in *Prdx6*$^{-/-}$ mice exposed to acute and chronic DSS. These results are in line with two others reports showing that in *Prdx6*$^{-/-}$ mice, the expression level of *Nrf2* and the total level of GSH was increased in ethanol-mediated oxidative stress in liver and in cigarette smoke-mediated lung inflammatory response and injury models (43, 58). Taken together, it appears that a lack of *Prdx6* leads to the activation of other antioxidant defense mechanisms that compensate and even overcompensate for the loss of *Prdx6* during DSS-mediated intestinal inflammation.

Although loss of *Prdx6* had significant anti-inflammatory effects in DSS-induced colitis, its overexpression did not affect colitis severity. Due to the protective effects of *Prdx6* deletion, one might expect enhanced colitis upon *Prdx6* overexpression. However, the protective effect upon loss of *Prdx6* seems to be due to compensatory mechanisms and seems to require enhanced expression of other peroxiredoxins. This might explain why an overexpression of *Prdx6* does not have the opposite effect on colitis severity as *Prdx6* deletion, in particular as we observed a likewise induction of Prdx3, Gss and Nrf2. The mechanisms how both loss as

well as overexpression of Prdx6 lead to increased expression of other Peroxiredoxins and antioxidant enzymes will need to be investigated in further studies.

In summary, the results of our study show, for the first time, that genetic ablation of *Prdx6* does not aggravate acute and chronic intestinal inflammation but is associated with increased expression of other peroxiredoxins and antioxidant enzymes/molecules, in particular *Nrf2* and its target genes *Gss*, *Gclm* and GSH. Our findings suggest that maintenance of cellular redox balance during DSS-induced acute and chronic intestinal inflammation by particular peroxiredoxins and antioxidant enzymes is critical. Downregulation of Prdx6 and compensatory up-regulation of Prdx3, Prdx4, Nrf2 and enzymes of the GSH pathway seem to be a physiologic response during experimental DSS colitis..

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Author contributions:

HM acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis; MRS acquisition of data; critical revision of the manuscript for important intellectual content; JCR acquisition of data; critical revision of the manuscript for important intellectual content; KA acquisition of data; analysis and interpretation of data; SL acquisition of data; analysis and interpretation of data; MS material support; critical revision of the manuscript for important intellectual content; GR study concept; analysis and interpretation of

data; critical revision of the manuscript for important intellectual content; IFW study concept and design; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; study supervision.

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Figure legends:

Figure 1. ***Prdx6*$^{-/-}$ mice are more resistant to DSS-induced acute colitis.**

Acute DSS colitis was induced by 2% DSS in the drinking water for seven days. Bodyweight of the animals was monitored daily and the percentage of the weight on day 0 is plotted as mean +/- **(A)** On day 7 colon length **(B)**, and MPO activity on homogenized colon samples **(C)** were determined. Prior to euthanization, colonoscopy was performed and pictures were scored using the murine endoscopic score of colitis severity (MEICS; **D).** Histologic assessment was performed on H&E stained distal colon tissue sections **(E)**. Representative pictures are shown in the right panel. **(F) Level of colon pro-inflammatory mediators in WT and *Prdx6*$^{-/-}$ mice.** Colon specimens of WT and KO mice after acute DSS administration and control samples were homogenized and the expression of *TNF, iNOS*, *IFN-γ, IL-6 and IL-1β* was studied by real-time RT-qPCR. Results are representative of 2 independent experiments per model and values are mean ± SEM (n = 12 mice per group). * $p < 0.05$.

Figure 2. ***Prdx6*$^{-/-}$ mice are more resistant to DSS-induced chronic colitis.**

Chronic DSS colitis was induced by five 7-day cycles of 2% DSS (indicated in yellow) interspaced with recovery phases with normal drinking water (indicated in white). Bodyweight of the animals was monitored daily (A). Four weeks after the last DSS cycle (day 105) colon length **(B)**, and MPO activity on homogenized colon samples **(C)** were determined. Prior to euthanization, colonoscopy was performed and evaluated by MEICS (**D).** Histologic assessment was performed on H&E stained distal colon tissue sections **(E)**. Representative pictures are shown in the right panel. **(F) Level of colon pro-inflammatory mediators in WT and *Prdx6*$^{-/-}$ mice.** Colon specimens of WT and KO mice after chronic DSS administration and control samples were homogenized and the expression of *TNF, iNOS*, *IFN-γ, IL-6 and IL-1β* was studied by real-time RT-qPCR. Results are representative of 2

692 independent experiments per model and values are mean ± SEM (n = 12 mice per group). * p
693 < 0.05; ** p < 0.01.
694
695 Figure 3. **Lower expression of Prdx6 in murine acute DSS colitis in contrast to**
696 **overexpression in human colonic biopsies of IBD patients**
697 Analysis of Prdx6 protein expression by Western blot in C57Bl/6 wildtype (A) and *Prdx6*$^{tg/tg}$
698 (B) mice. Results are representative of 2 independent experiments per model with n = 12
699 mice per group and values are given as mean ± SEM. * p < 0.05; *** p < 0.001.
700 Total RNA was isolated from human colonic and ileal biopsies of IBD patients and controls,
701 reversely transcribed and the expression of *Prdx6* versus *Vil1* was studied by real-time RT-
702 qPCR (D, E) (colon: healthy control n = 8, CD non-inflamed n = 15, CD inflamed n = 18, CD
703 remission n = 5, UC non-inflamed n = 20, UC inflamed n = 18, UC remission n = 2; ileum:
704 healthy control n = 13, CD non-inflamed n = 6, CD inflamed n = 4, CD remission n = 3, UC
705 non-inflamed n = 4, UC inflamed n = 1, UC remission n = 2
706
707 Figure 4. **Increased mRNA and protein expression of Peroxiredoxins and antioxidant**
708 **enzymes and increased GSH-levels in *Prdx6*$^{-/-}$ mice exposed to DSS.**
709 (A and B) mRNA expression of *Prdx3*, *Prdx4* and the antioxidant enzymes *Nrf2*, *Gss* and
710 *Gclm* were determined by RT-qPCR relative to β-actin in colon tissue samples from WT and
711 *Prdx6*$^{-/-}$ mice exposed to acute and chronic DSS. (C and D) Western blot analysis of *Prdx3*,
712 *Prdx4*, *Nrf2*, *Gss*, *Gclm* and *Gclc* obtained from colonic samples of WT mice and *Prdx6*$^{-/-}$
713 mice exposed to acute and chronic DSS (E and F). Colon levels of total GSH in WT and
714 *Prdx6*$^{-/-}$ mice exposed to acute and chronic DSS administration. Total GSH was quantified by
715 measuring the absorbance of TNB and GS-TNB adducts at 412 nm in colon homogenates.
716 Results are representative of 2 independent experiments per model and values are mean ±
717 SEM (n = 12 mice per group). * p < 0.05; ** p < 0.01, *** p < 0.001.

718

719 Figure 5**. Increased expression of Prdx3, Nrf2 and Gss in LPMC but not in IEC**

720 Analayses of mRNA expression of *Prdx3*, *Prdx4* and the antioxidant enzymes *Nrf2*, *Gss* and
721 Gclm in epithelial cells and in lamina propria mononuclear cells isolated form in colon tissue
722 samples from WT and *Prdx6-/-* mice exposed to acute and chronic DSS.

723

724 Figure 6**. Overexpression of *Prdx6* does not influence the intestinal inflammation in acute**
725 **DSS colitis.**

726 Acute DSS colitis was induced by 2% DSS in the drinking water for seven days. Bodyweight
727 of the animals was monitored daily and the percentage of the weight on day 0 is plotted as
728 mean +/- **(A)** On day 7 colon length **(B)**, and MPO activity on homogenized colon samples
729 **(C)** were determined. Prior to euthanization, colonoscopy was performed and pictures were
730 scored using the murine endoscopic score of colitis severity (MEICS; **D).** Histologic
731 assessment was performed on H&E stained distal colon tissue sections **(E)**. Representative
732 pictures are shown in the right panel. Colon specimens of WT and KO mice after acute DSS
733 administration and control samples were homogenized and the expression of *TNF, iNOS*,
734 *IFN-γ, IL-6 and IL-1β* was studied by real-time RT-qPCR (**F**). Results are representative of 2
735 independent experiments per model and values are mean ± SEM (n = 12 mice per group)

736

737 Figure 7. **Overexpression of *Prdx6* does not affect the inflammation in chronic DSS colitis**
738 Chronic DSS colitis was induced by five 7-day cycles of 2% DSS (indicated in yellow)
739 interspaced with recovery phases with normal drinking water (indicated in white).
740 Bodyweight of the animals was monitored daily **(A)**. Four weeks after the last DSS cycle (day
741 105) colon length **(B)**, and MPO activity on homogenized colon samples **(C)** were
742 determined. Prior to euthanization, colonoscopy was performed and evaluated by MEICS (**D).**
743 Histologic assessment was performed on H&E stained distal colon tissue sections **(E)**.

744 Representative pictures are shown in the right panel. Colon specimens of WT and KO mice
745 after chronic DSS administration and control samples were homogenized and the expression
746 of *TNF, iNOS* and *IFN-γ* was studied by real-time RT-qPCR (**F**). Results are representative of
747 2 independent experiments per model and values are mean ± SEM (n = 12 mice per group). *
748 $p < 0.05$.
749
750 Figure 8. **Increased expression of Prdx3, Gss and Nrf2 in colons of WT and *Prdxg*$^{tg/tg}$**
751 **mice exposed to chronic DSS**
752 mRNA expression of *Prdx3*, *Prdx4* and the antioxidant enzymes *Nrf2*, *Gss* and *Gclm* were
753 determined by RT-qPCR relative to β-actin in colon tissue samples from WT and *Prdx6*$^{tg/tg}$
754 mice exposed to chronic DSS.
755

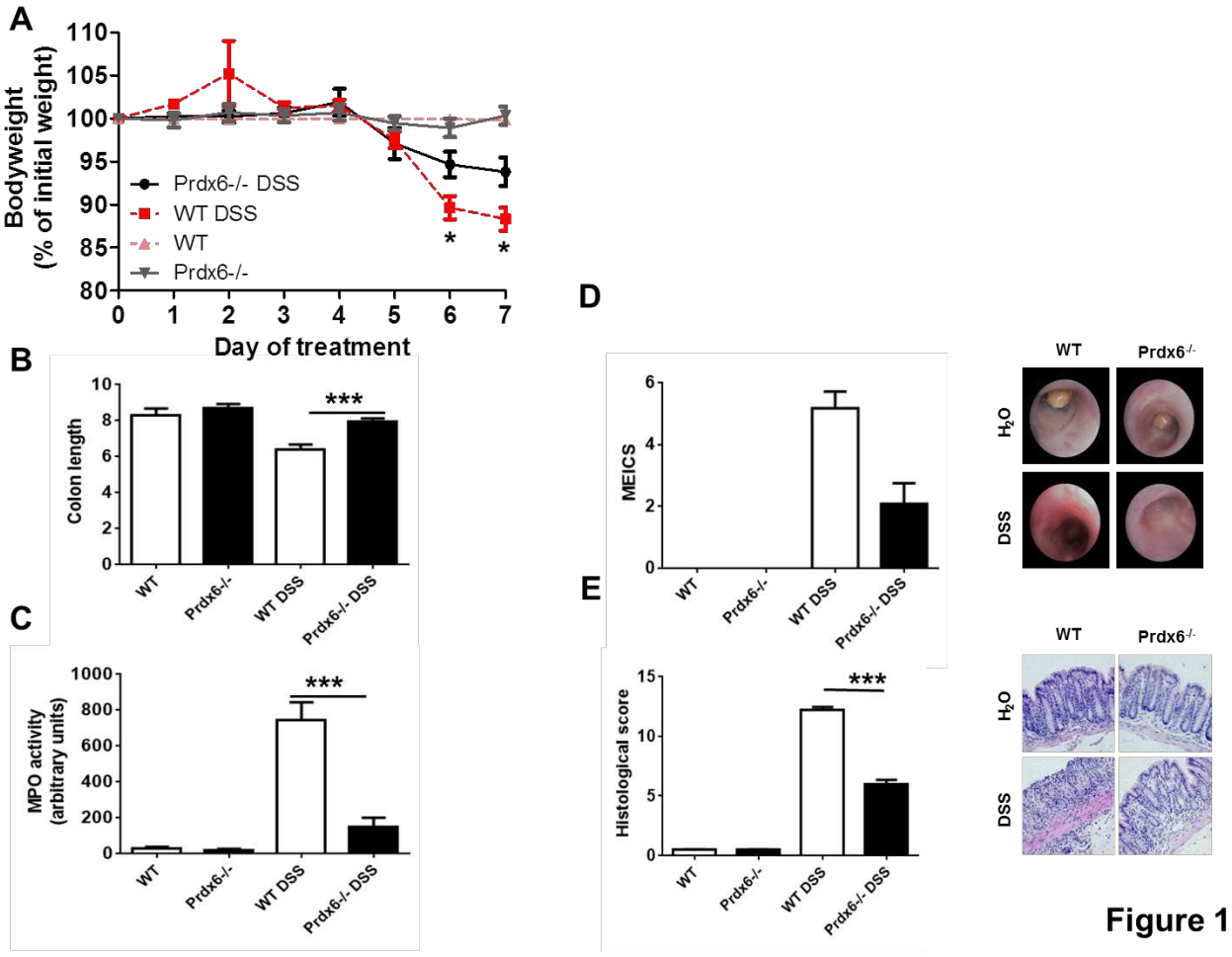
A
Bodyweight (% of initial weight)
Day of treatment
Prdx6-/- DSS
WT DSS
WT
Prdx6-/-
B
Colon length
C
MPO activity (arbitrary units)
D
MEICS
E
Histological score
WT
Prdx6-/-
WT DSS
Prdx6-/- DSS
Prdx6-/-
H2O
DSS

Figure 1

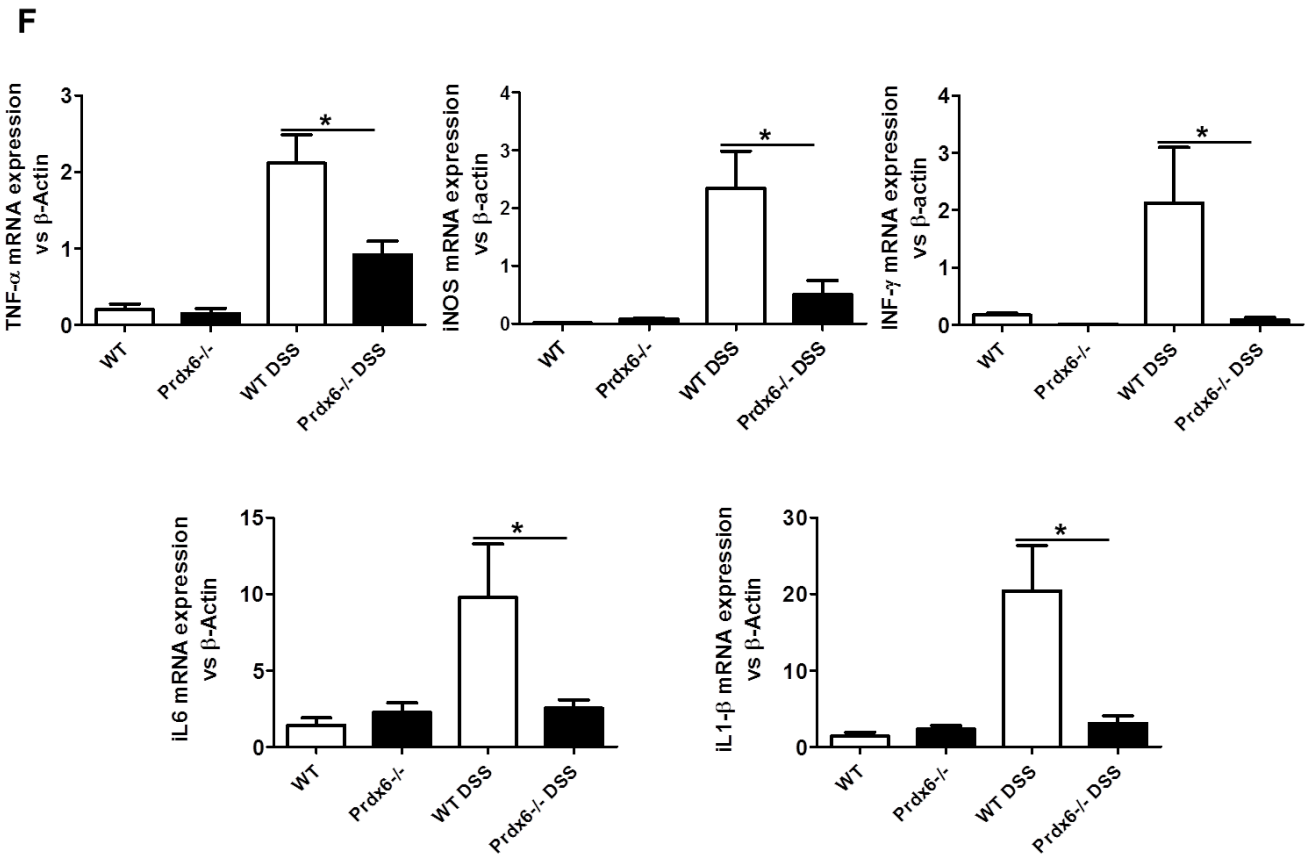
F
TNF-α mRNA expression vs β-Actin
iNOS mRNA expression vs β-actin
INF-γ mRNA expression vs β-actin
iL6 mRNA expression vs β-Actin
iL1-β mRNA expression vs β-Actin
WT
Prdx6-/-
WT DSS
Prdx6-/- DSS
*

Figure 1

757

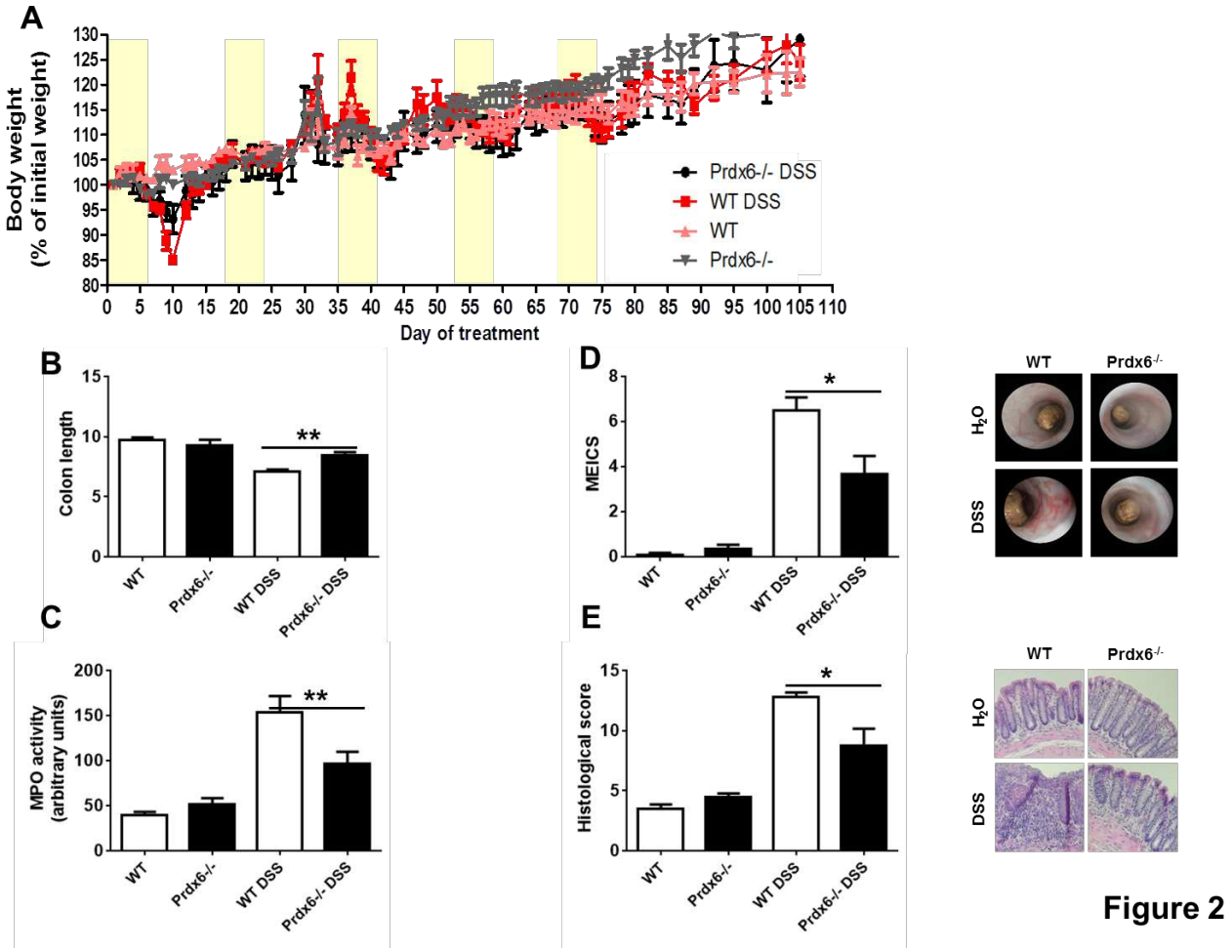
A
Body weight (% of initial weight)
Day of treatment
Prdx6-/- DSS
WT DSS
WT
Prdx6-/-
B
Colon length
C
MPO activity (arbitrary units)
D
MEICS
E
Histological score
WT
Prdx6-/-
WT DSS
Prdx6-/- DSS
H2O
DSS
Prdx6-/-

Figure 2

758

F

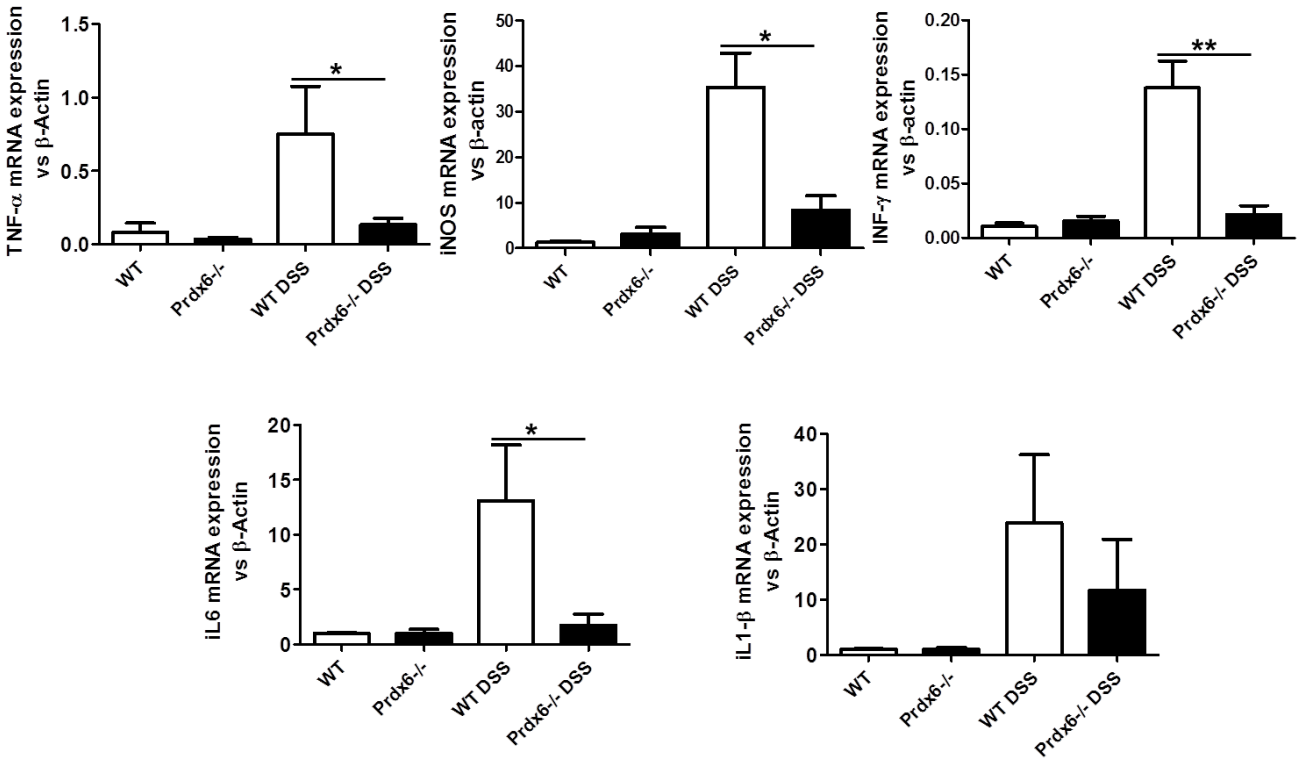

Figure 2

759

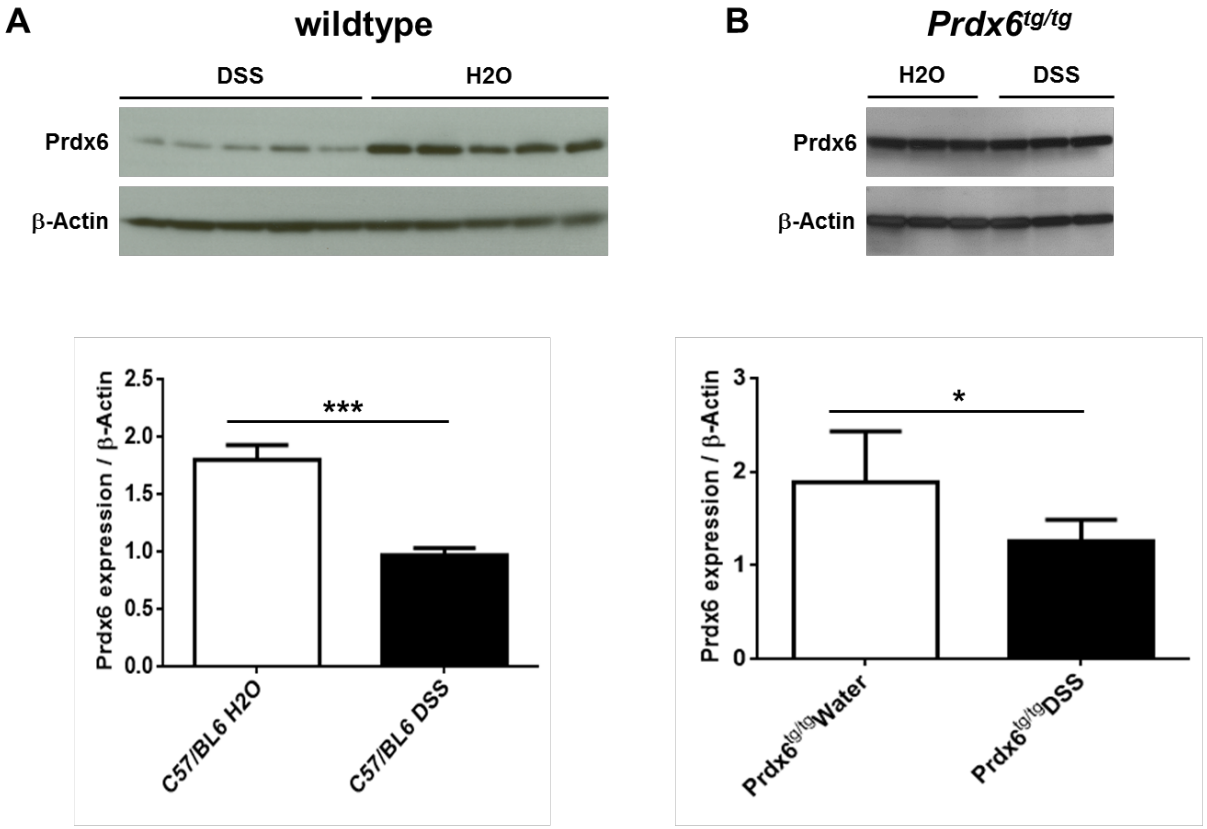
A
wildtype
DSS
H2O
Prdx6
β-Actin
B
Prdx6tg/tg
H2O
DSS
Prdx6
β-Actin
Prdx6 expression / β-Actin
2.5
2.0
1.5
1.0
0.5
0.0
***
C57/BL6 H2O
C57/BL6 DSS
Prdx6 expression / β-Actin
3
2
1
0
*
Prdx6tg/tgWater
Prdx6tg/tgDSS

Figure 3

760

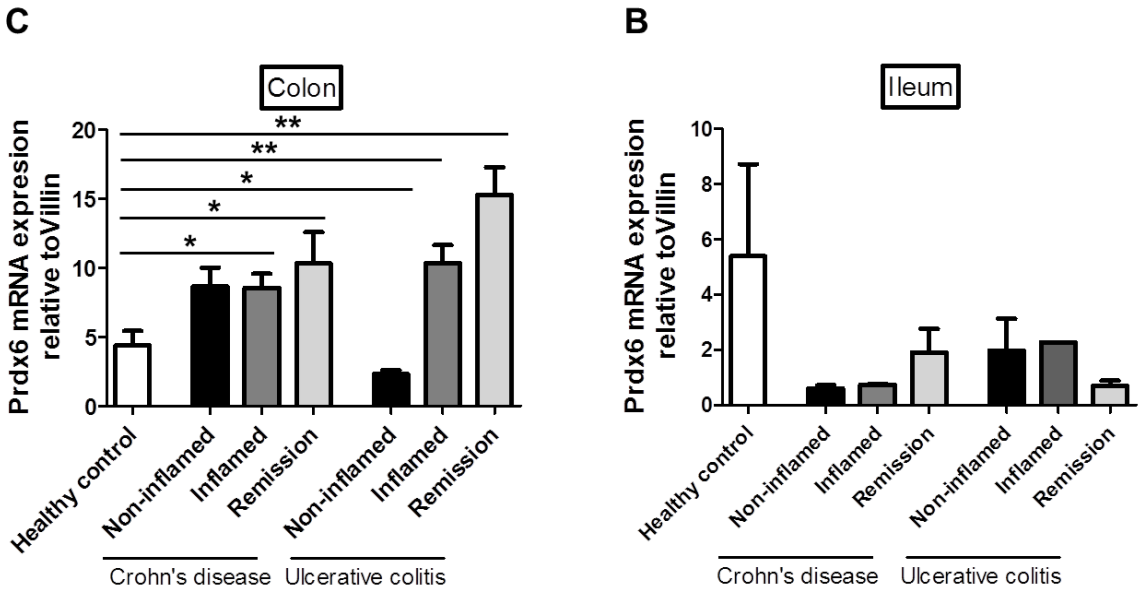
C
Colon
Prdx6 mRNA expresion relative toVillin
20
15
10
5
0
**
**
*
*
*
Healthy control
Non-inflamed
Inflamed
Remission
Non-inflamed
Inflamed
Remission
Crohn's disease
Ulcerative colitis
B
Ileum
Prdx6 mRNA expresion relative toVillin
10
8
6
4
2
0
Healthy control
Non-inflamed
Inflamed
Remission
Non-inflamed
Inflamed
Remission
Crohn's disease
Ulcerative colitis

Figure 3

761

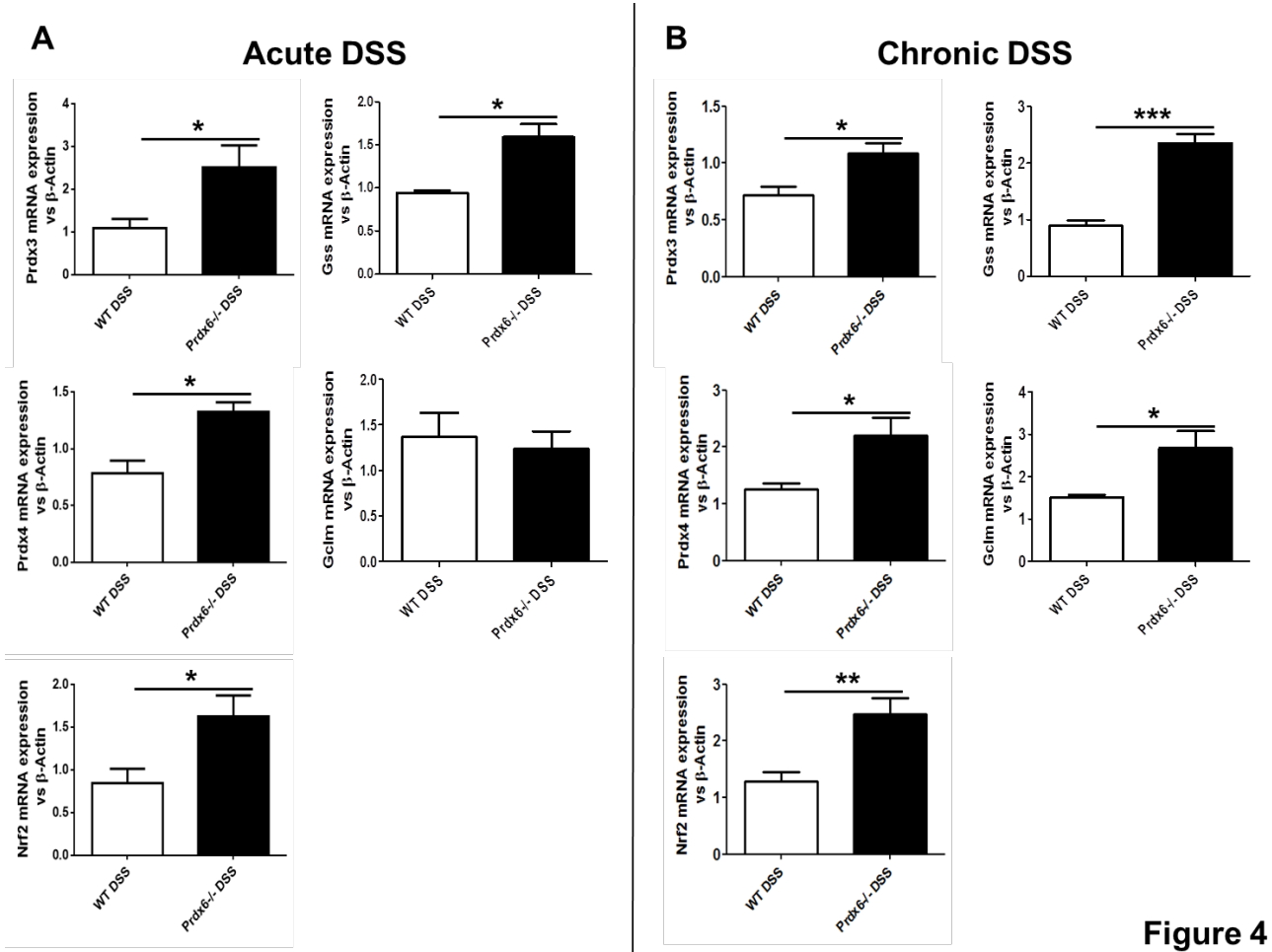
A
Acute DSS
Prdx3 mRNA expression vs β-Actin
Gss mRNA expression vs β-Actin
Prdx4 mRNA expression vs β-Actin
Gclm mRNA expression vs β-Actin
Nrf2 mRNA expression vs β-Actin
WT DSS
Prdx6-/- DSS
B
Chronic DSS
Prdx3 mRNA expression vs β-Actin
Gss mRNA expression vs β-Actin
Prdx4 mRNA expression vs β-Actin
Gclm mRNA expression vs β-Actin
Nrf2 mRNA expression vs β-Actin
WT DSS
Prdx6-/- DSS

Figure 4

762

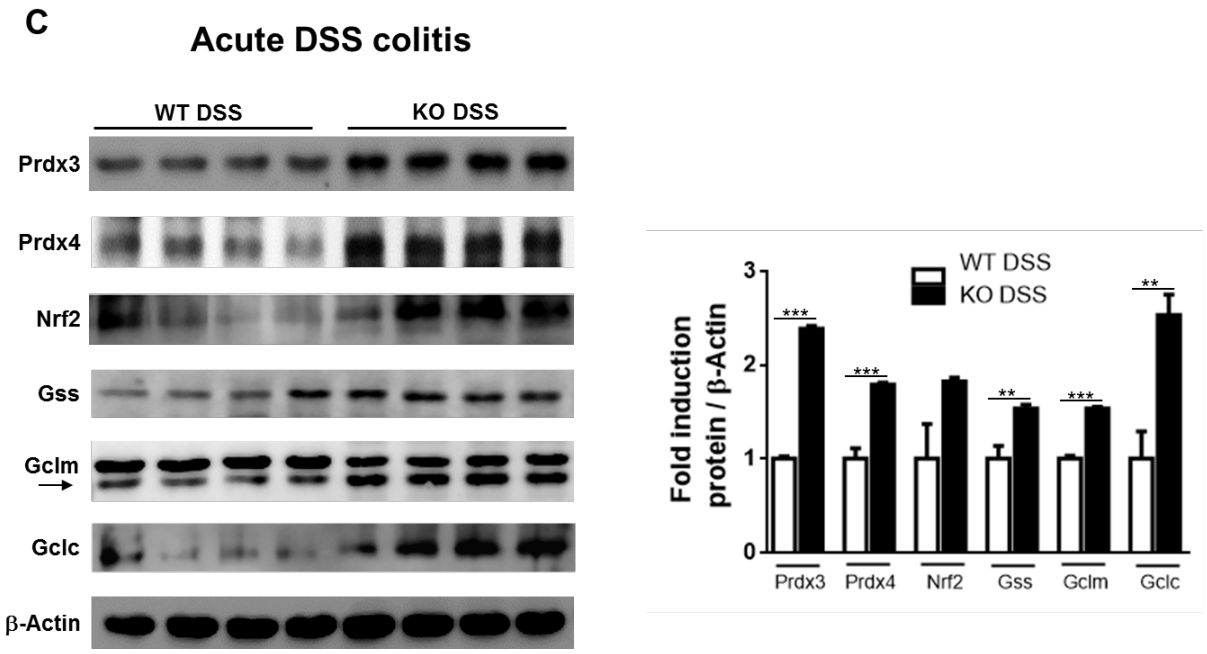
C
Acute DSS colitis
WT DSS
KO DSS
Prdx3
Prdx4
Nrf2
Gss
Gclm
Gclc
β-Actin
WT DSS
KO DSS
Fold induction protein / β-Actin
0
1
2
3
***
***
**
***
**
Prdx3
Prdx4
Nrf2
Gss
Gclm
Gclc

Figure 4

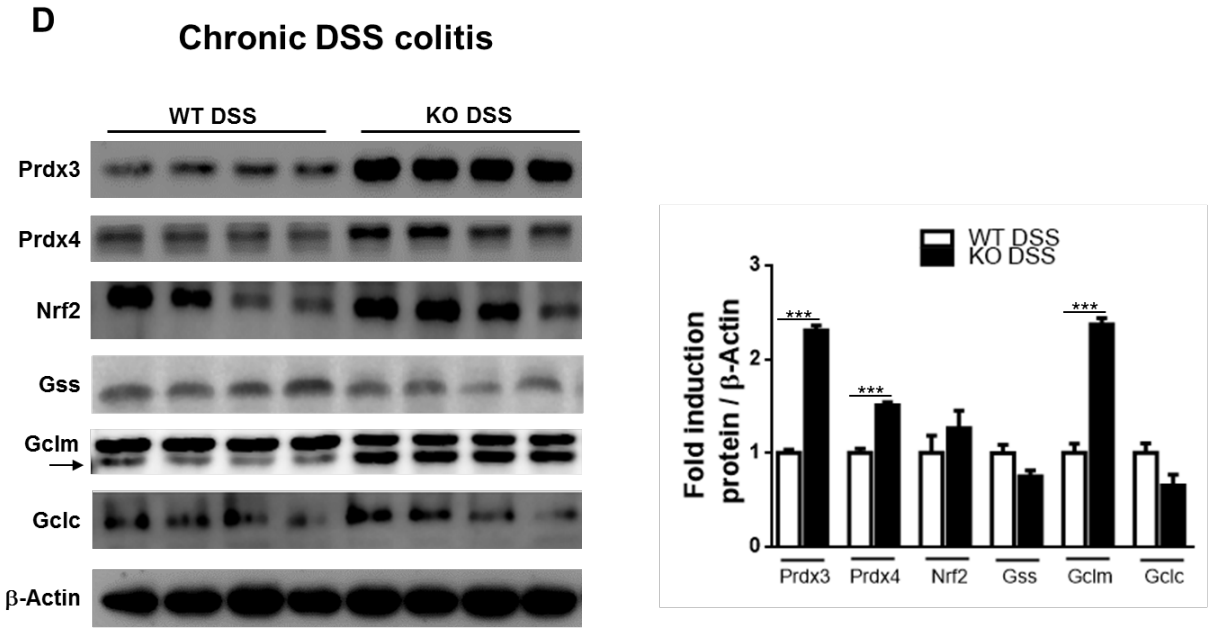
D
Chronic DSS colitis
WT DSS
KO DSS
Prdx3
Prdx4
Nrf2
Gss
Gclm
Gclc
β-Actin
WT DSS
KO DSS
Fold induction protein / β-Actin
0
1
2
3
***
***
***
Prdx3
Prdx4
Nrf2
Gss
Gclm
Gclc

Figure 4

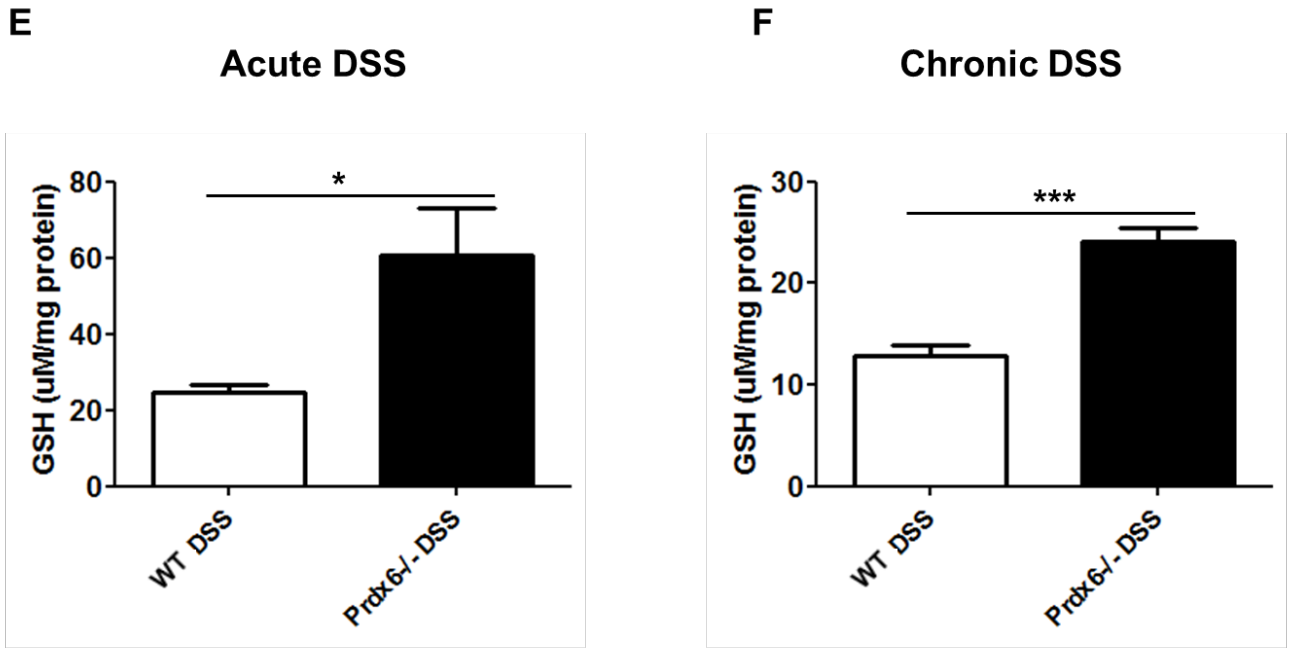
E
Acute DSS
GSH (uM/mg protein)
80
60
40
20
0
*
WT DSS
Prdx6-/- DSS
F
Chronic DSS
GSH (uM/mg protein)
30
20
10
0
***
WT DSS
Prdx6-/- DSS

Figure 4

765

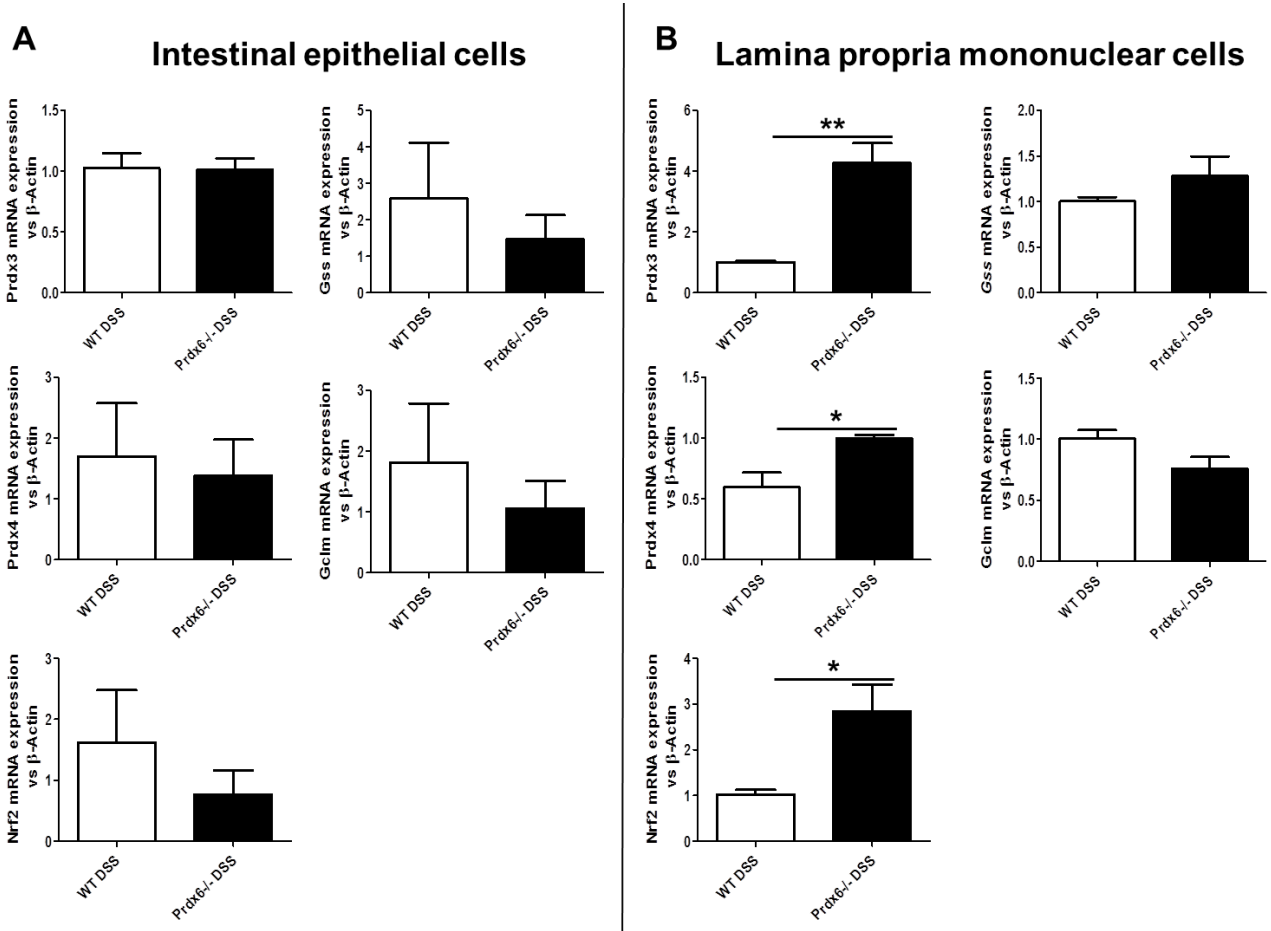
A
Intestinal epithelial cells
B
Lamina propria mononuclear cells
Prdx3 mRNA expression vs β-Actin
Gss mRNA expression vs β-Actin
Prdx4 mRNA expression vs β-Actin
Gclm mRNA expression vs β-Actin
Nrf2 mRNA expression vs β-Actin
WT DSS
Prdx6-/- DSS
**
*
*

Figure 5

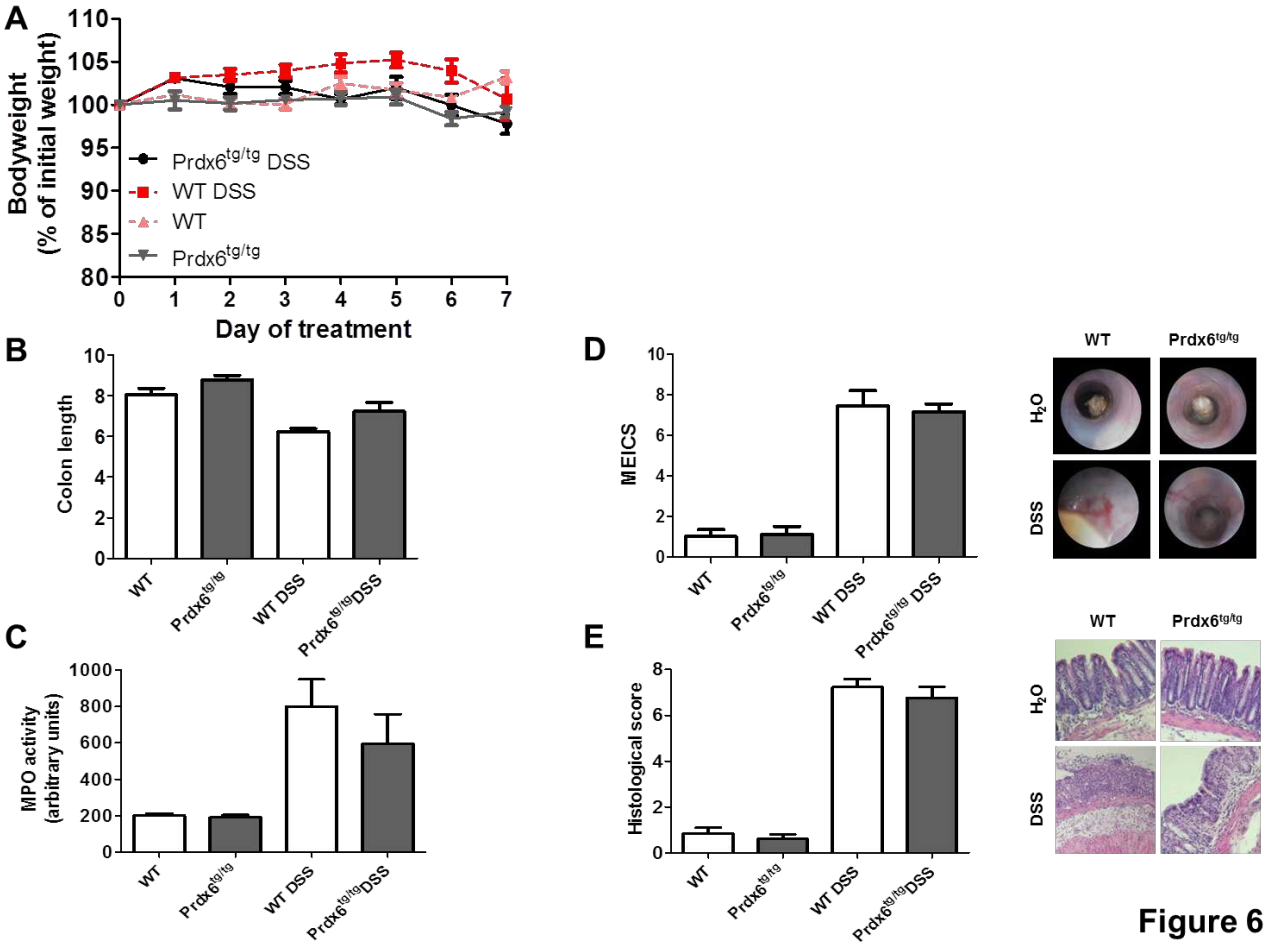
A
Bodyweight (% of initial weight)
110
105
100
95
90
85
80
0 1 2 3 4 5 6 7
Day of treatment
Prdx6tg/tg DSS
WT DSS
WT
Prdx6tg/tg
B
Colon length
10
8
6
4
2
0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS
C
MPO activity (arbitrary units)
1000
800
600
400
200
0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS
D
MEICS
10
8
6
4
2
0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tg DSS
E
Histological score
8
6
4
2
0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS
WT
Prdx6tg/tg
H2O
DSS
WT
Prdx6tg/tg
H2O
DSS

Figure 6

F

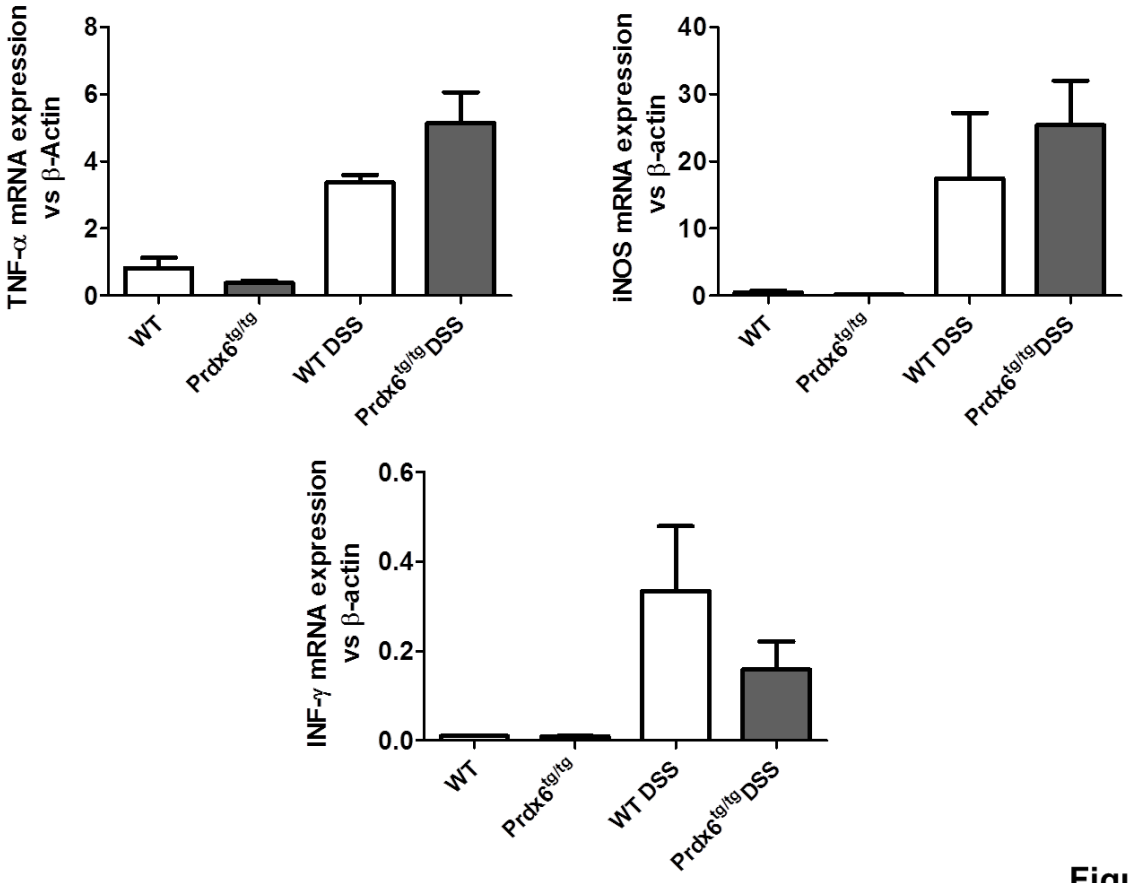
TNF-α mRNA expression vs β-Actin
8
6
4
2
0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS
iNOS mRNA expression vs β-actin
40
30
20
10
0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS
INF-γ mRNA expression vs β-actin
0.6
0.4
0.2
0.0
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS

Figure 6

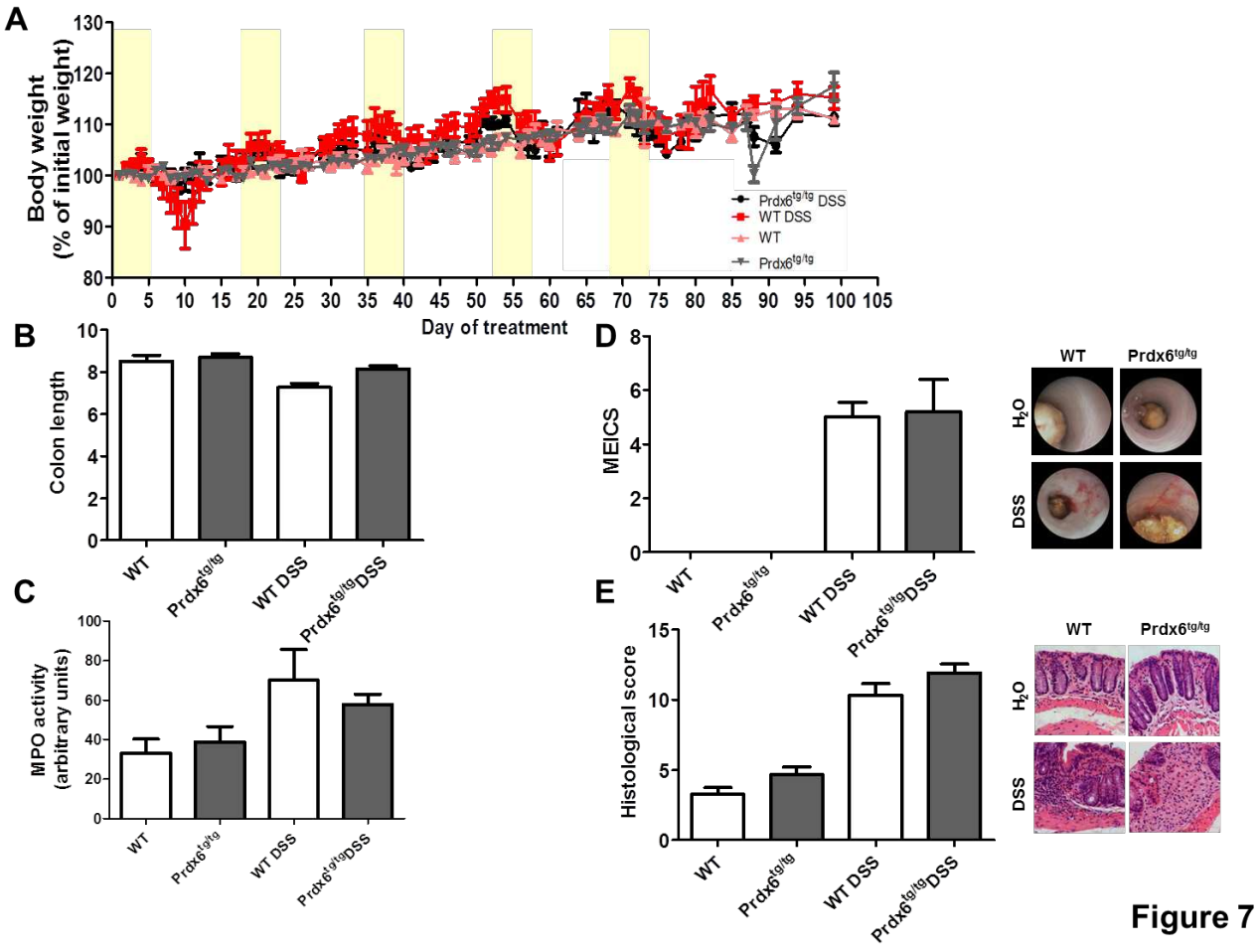
A
Body weight (% of initial weight)
Day of treatment
Prdx6tg/tg DSS
WT DSS
WT
Prdx6tg/tg
B
Colon length
C
MPO activity (arbitrary units)
D
MEICS
E
Histological score
WT
Prdx6tg/tg
WT DSS
Prdx6tg/tgDSS
H2O
DSS

Figure 7

F

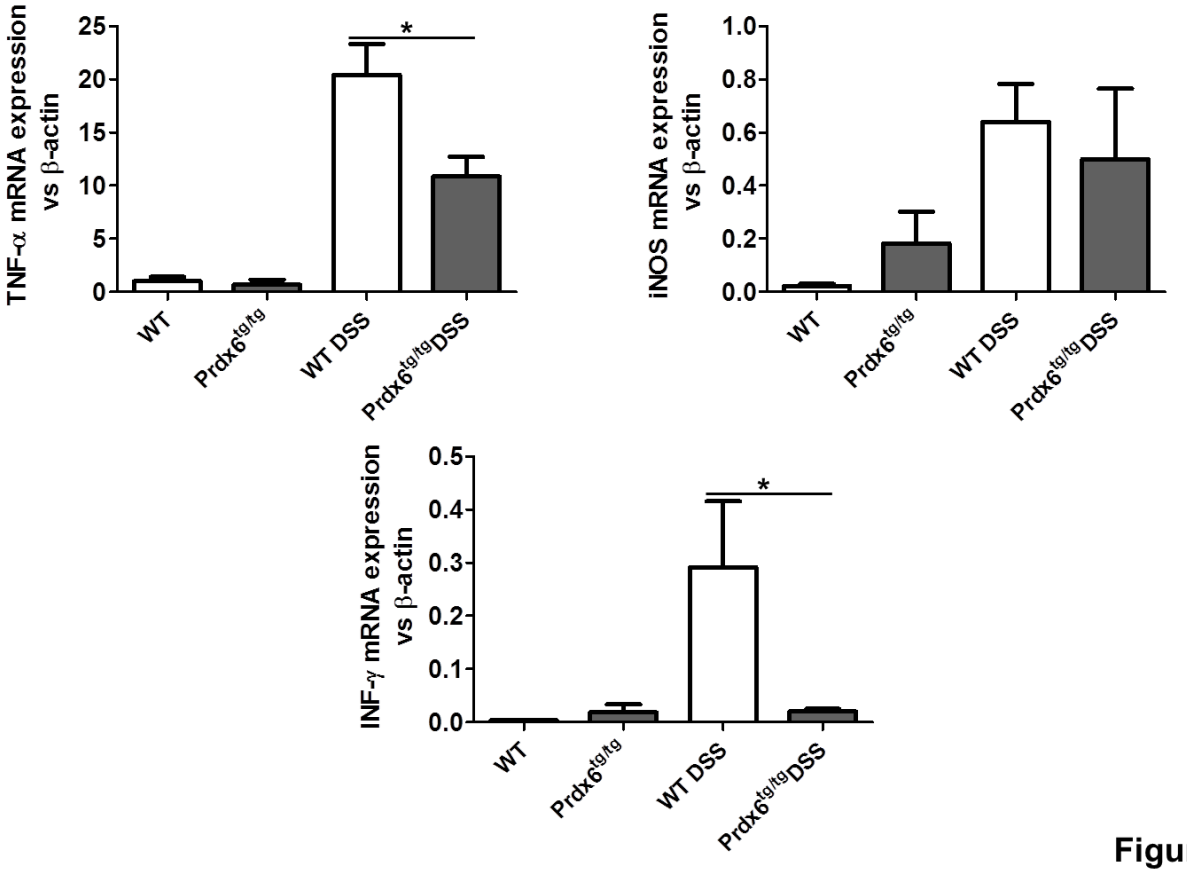

Figure 7

770

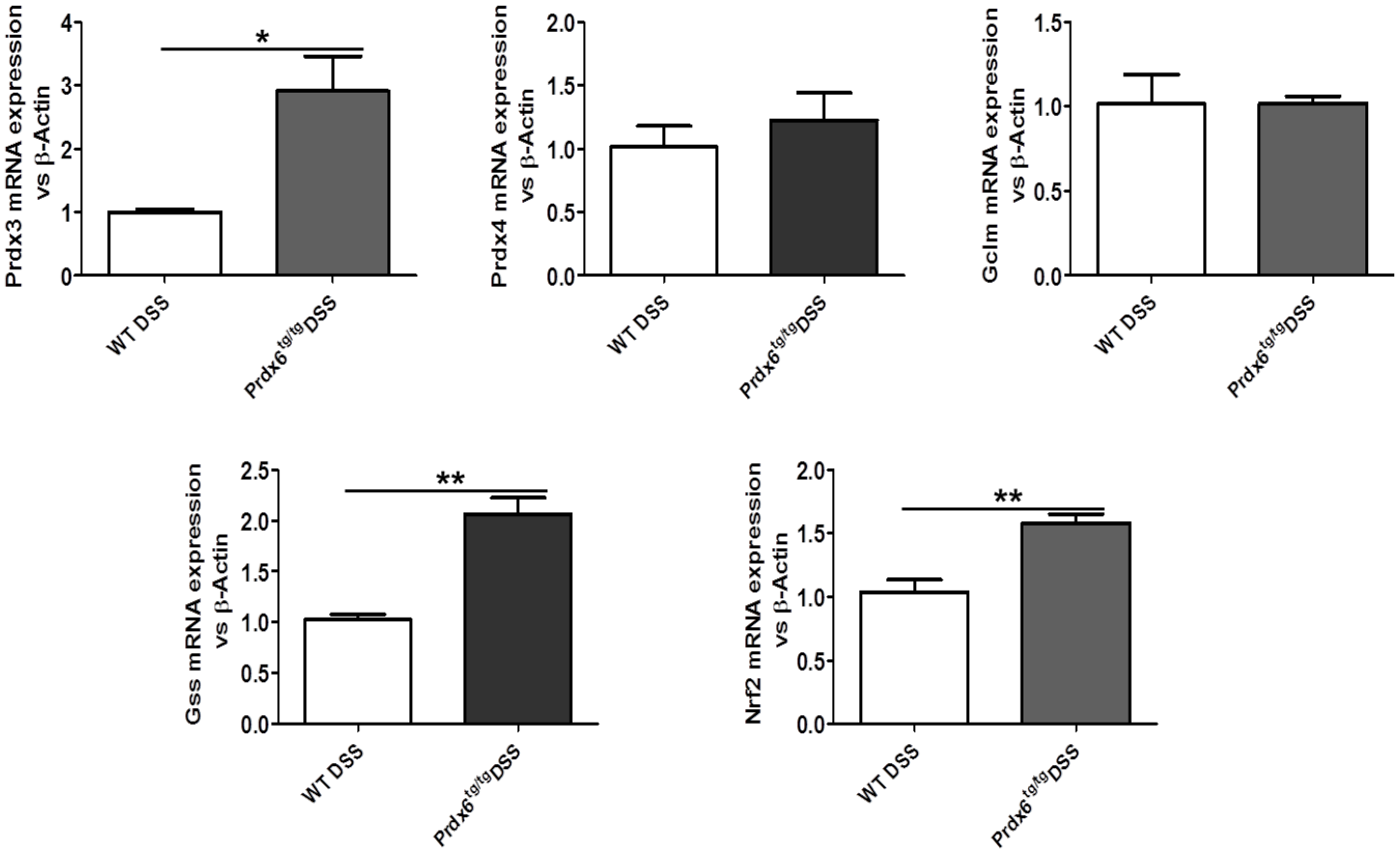
Prdx3 mRNA expression vs β-Actin
*
WT DSS
Prdx6tg/tgDSS
Prdx4 mRNA expression vs β-Actin
WT DSS
Prdx6tg/tgDSS
Gclm mRNA expression vs β-Actin
WT DSS
Prdx6tg/tgDSS
Gss mRNA expression vs β-Actin
**
WT DSS
Prdx6tg/tgDSS
Nrf2 mRNA expression vs β-Actin
**
WT DSS
Prdx6tg/tgDSS

Figure 8

771